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IFN-γ, IL-10, ghrelin and nesfatin-1 levels in acute brucella patients

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Abstract

Aim: This study aimed to investigate gamma interferon (IFN-γ), interleukin-10 (IL-10), ghrelin and nesfatin-1 levels in acute brucella patients and to evaluate the effect of combination of antimicrobial agents after therapy.

Material and Methods: Patients were assigned to the Brucella-infected group if they had confirmed laboratory findings. Sixty brucella-infected patients and thirty healthy individuals were included in the study. Doxycycline-streptomycin (DOX-STR) and doxycycline-rifampicin (DOX-RIF) were used in the treatment of brucellosis.

Results: Significantly increased IFN-γ, IL-10 and ghrelin levels and decreased nesfatin-1 levels were determined in brucella patients compared with healthy controls ($p < 0.001$). After therapy, IFN-γ, IL-10 and ghrelin levels were significantly lower, and nesfatin-1 levels were significantly higher compared to the pre-treatment group, especially in (DOX-STR) regimen ($p < 0.001$).

Discussion: It is thought that the rise of free oxygen radicals and the decrease in antioxidant capacity may play a role in the pathogenesis of brucellosis. In this study, the therapeutic power of antibiotic combinations was determined by monitoring inflammatory agents and ghrelin and nesfatin-1 levels measured before and after treatment in patients. Adding ghrelin and nesfatin-1 to effective treatment protocols for brucella will ameliorate their inflammatory status and antioxidant system.

Keywords

Brucellosis, Gamma Interferon (IFN-γ), Interleukin-10 (IL-10), Ghrelin, Nesfatin-1, Antibiotic treatment

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Introduction

Brucellosis is a systemic and inflammatory disease caused by the brucella bacteria, which are gram (-) coccobacillus. It is an important public health problem and especially common in Turkey or developing countries such as the Mediterranean basin of Europe, the Middle East of Asia, and South and Central America. It has a zoonotic character (WHO. Brucellosis fact sheet N173. Geneva, Switzerland: WHO; 1997. p.23-37.); therefore, brucella agents infect humans through direct contact with infected animals or by eating their products. Transmission from animals to humans is commonly caused by Brucella melitensis (sheep and goats) and less often by Brucella abortus (cattle). Symptoms of the disease include loss of appetite and weight, bumpy fever, sweating, fatigue, joint and back pain. In chronic cases, it also causes arthritis, spondylitis, endocarditis, orchitis and osteomyelitis [1].

Many studies have shown that in inflammatory diseases, various of inflammatory cells are activated and cytokines are secreted, which leads to the over-production of reactive oxygen species (ROS) to kill intracellular pathogens. It has been found that an increment of free radical production and antioxidant depletion, and oxidative stress plays an important role in the pathogenesis of brucellosis [2,3]. It has been postulated that pro-inflammatory Th1 cytokines such as gamma interferon (IFN-γ) and interleukin-12 (IL-12) regulate the initiation of the adaptive immune response against brucellosis and protect the host. Anti-inflammatory Th2 cytokines such as IL-4 and IL-10 support the survival of bacteria and may facilitate pathogenesis [3].

Eating disorders, obesity and cachexia threaten millions of people around the world. Physiologically, the organism's short-term food intake control is regulated by the gastrointestinal tract, hypothalamus, and obesity hormones such as leptin, adinopectin, resistin, ghrelin, and nesfatin-1 released from many parts of the body. It is thought that ghrelin and nesfatin-1 play a key role in the regulation of appetite, food intake, energy expenditure, and body weight in the short and long term [4,5]. Ghrelin, first described in 1999 by Kojima et al., is a peptide hormone that is produced by the gastrointestinal system in the human body and plays a central role in the regulation of appetite and body weight. Ghrelin is secreted in an inactive, deacylated form, when ghrelin is acylated, thereby converting to the active form. It has been determined that low ghrelin levels increase calorie intake, leading to obesity or high ghrelin levels are determined in fasting state cachexia and anorexia nervosa [4]. Nesfatin-1, on the other hand, is an anoroxygenic peptide that is formed as a result of the breakdown of a precursor protein called NUCB2, which consists of 82 amino acids synthesized from the hypothalamus and is responsible for regulating appetite and producing body fat. The following studies have reported that excessive nesfatin-1 levels in the brain cause loss of appetite decrease in body fat and weight [5]. In the clinical picture of Brucella, symptoms such as fatigue, loss of appetite and weakness are observed. These studies show that ghrelin and nesfatin-1 may have an effect on diseases such as brucella, which progress with weight loss.

In this study, IFN-γ and IL-10 levels, as indicators of inflammatory agents, and ghrelin and nesfatin-1 levels, which are at different levels in cases such as obesity, weight and appetite loss, and weakness, will be elucidated with healthy controls in acute brucella patients. In addition, it will be tried to determine the therapeutic power of antibiotic combinations used in the therapy of brucellosis by measuring levels before and after treatment.

Material and Methods

Sixty brucella-infected patients and 30 healthy controls attending the outpatient Clinic of Infectious Diseases at the Cankiri State Hospital were included in the study. The study was approved by the Research Ethics Committee of the University of Zonguldak Karaelmas/Turkey. The age range and gender in brucella patients and the control group were 18 – 50 years, 32 men and 28 women / 19 – 45 years, 15 men and 15 women, respectively. Table 1 shows the demographic features of individuals and no statistically significant difference was found between the groups (age, gender, body mass index (BMI), BMI score, and waist circumference).

Patients were assigned to the Brucella-infected group if they had an epidemiological history (contact with animals and animal products), clinical symptoms (hyperhidrosis, undulant fever and joint pain, etc.), and confirmed laboratory findings (determination with blood culture, serum agglutination test [SAT] with titres ≥ 1/160). Exclusion criteria of the study for the patient and control group were pregnancy, drug use, alcohol or tobacco use, obesity (BMI ≥ 30), gastro/metabolic/inflammatory diseases, abdominal surgery.

As ghrelin and nesfatin-1 are peptide hormones, aprotinin (500 Kallikrein units/mL) was put into the blood tubes before the study to protect against serum proteases. Before and after 45 days of antibiotic treatment, blood specimens (5mL) were collected from the participants by venipuncture. The serum samples of 2 mL were stored at –70 °C until further analysis of IFN-γ, IL-10, ghrelin and nesfatin-1 levels.

Serum samples were analyzed for IFN-γ and IL-10 using human enzyme-linked immunosorbent assay (ELISA) kits (Bender MedSystems GmbH, Austria) and the absorbance was determined at 450 nm using a microplate reader (BioTek Microplate Instruments, USA). Serum acylated, desacylated ghrelin and nesfatin-1 levels were evaluated using human ELISA kits (Cat. No: A05106/A05119, SPI-BIO, France; Cat. no. EIA-NES-1, RayBiotech Inc., Georgia). The absorbance values were read at 410 nm and 450 nm using a microplate reader (BioTek Microplate Instruments, USA), spectrophotometrically.

Finally, the mathematical sum of total ghrelin levels with acyl and desacyl ghrelin values was calculated. The same trade kits were used in the determination of acyl and desacyl ghrelin levels so that the total can be calculated correctly.

Statistical Analysis

While evaluating the findings obtained in the study, SPSS program (Statistical Package for Social Sciences 13.0, USA) was used for statistical analysis. The Mann-Whitney-U test and Spearman's correlation analysis were used to compare the differences between groups. The p-value was 0.001 and all data in the tables were mean ± SD.

Results

In brucellosis, antibiotic combinations such as doxycycline-streptomycin (DOX-STR) and doxycycline-rifampicin (DOX-RIF) are commonly used in treatment (Joint FAO/WHO Expert Committee on Brucellosis. Joint FAO/WHO Expert Committee on Brucellosis: sixth report. WHO Technical Report Series No. 740. Geneva; 1986.p.56-7.). Treatment regimens for patients with brucellosis in this study included 200 mg/day orally doxycycline plus 1 g/day intramuscularly streptomycin for 45 days or 200 mg/day orally doxycycline plus 600 mg/day orally rifampicin for 45 days. Hematological and biochemical parameters at pre- and post-therapy are given in Table 2.

The cytokine, ghrelin and nesfatin-1 parameters for all participants are shown in Table 3. Significantly higher IFN-γ and IL-10 levels were found in the acute brucellosis study group than in controls ($p < 0.001$). When the two groups were compared based on ghrelin levels, there was a statistically significant increment between the groups ($p < 0.001$). Significantly decreased nesfatin-1 levels were found in brucellosis patients compared with controls ($p < 0.001$).

Table 3 also shows outcome data for patients treated with the antibiotic combination DOX-STR or DOX-RIF. When comparing two treatments with the patient group, the cytokine status, ghrelin and nesfatin-1 levels of the patients normalized after the treatment period. When comparing the two treatment regimens, there was no significant difference ($p> 0.05$). Comparison of different antibiotic combinations showed the benefit of using DOX-STR.

Table 1. Demographic features of the control and patient groups

	Control	Patients
N	30	60
Gender (M/F)	15/15	32/28
Age* (year)	33.2 ± 8.1	34.3 ± 8.7
BMI* (kg/m2)	23.5 ± 4.2	22.8 ± 2.7
BMI score*	2.03 ± 0.35	1.97 ± 0.41
Waist circumference*	89.42 ± 8.22	87.32 ± 7.15

*Mean ± standard deviation; P-value: p>0,05

Discussion

Brucellosis is a disease caused by Brucella spp and these bacteria are taken into the cell by phagocytic cells. During the normal immune response, macrophages and dendritic cells are activated for antigen processing, leading to the production of cytokines. Pro-inflammatory Th1 cytokines such as gamma interferon (IFN-γ) and interleukin-12 (IL-12) activate macrophages to halt the replication of intracellular brucellae and to produce ROS to kill brucella spp. It is thought that the rise of free oxygen radicals and the decrease of antioxidant

Table 2. Hematological and biochemical findings of the control and patient groups

	After 45 days of treatment with			
Variables	**Control**	**Patients**	**(DOX-STR)**	**(DOX-RIF)**
Red blood cells (×10⁶/μL)	4.31 ± 0.84	4.37 ± 0.61	4.41 ± 0.47	4.33 ± 0.52
White blood cells (×10³/μL)	6.92 ± 2.9	8.80 ± 3.2*	8.25 ± 5.3*	7.36 ± 4.8*
Total bilirubin (mg/dL)	0.71 ± 0.18	0.63 ± 0.13*	0.65 ± 0.22*	0.68 ± 0.35*
Albumin (g/dL)	4.04 ± 0.37	3.70 ± 1.9	4.11 ± 0.8**	3.92 ± 1.3**
Glucose (mg/dL)	87.35 ± 11.75	111.19 ± 31.16*	105.22 ± 32.41*	106.34 ± 24.46*
Creatinine (mg/dL)	0.94 ± 0.21	0.70 ± 0.17*	0.84 ± 0.22**	0.81 ± 0.25**
C-reactive protein (mg/L)	5.2 ± 1.4	30 ± 3.85*	9 ± 1.67**	10 ± 1.95**
Aspartate amino transferase (IU/L)	25.2 ± 7.4	48.0 ± 9.34*	28.2 ± 8.42**	32.7 ± 7.26**
Alanine aminotransferase (IU/L)	27.7 ± 8.3	42.0 ± 7.2*	31.3 ± 6.31**	36.4 ± 5.98**
Lactate dehydrogenase (IU/L)	583 ± 124.6	540 ± 142.7*	564 ± 131.8	572 ± 99.1**
Gamma glutamyl transferase (IU/L)	65.32 ± 24.1	70 ± 17.3*	64.27 ± 31.8**	67.14 ± 19.7
Alkaline phosphatase (IU/L)	115.45 ± 14.5	310 ± 16.4*	195.2 ± 27.8*,**	228 ± 13.9*,**
Triglycerides (mg/dL)	125.81 ± 74.3	134.65 ± 62.8	127.38 ± 21	132.43 ± 35.6
HDL (mg/dL)	58 ± 5.6	42 ± 4.9*	51 ± 3.8*,**	49 ± 2.7*,**
LDL (mg/dL)	125.3 ± 25.3	155.4 ± 14.5*	133.2 ± 35.6*,**	145.2 ± 19.1*,**
Total cholesterol (mg/dL)	178.24 ± 19.43	207.32 ± 22.7*	171.12 ± 15.76**	176.19 ± 13.52**

Results are represented as mean ± SD, *P < 0.001, significant compared with controls; **P < 0.001, significant compared with patients

Table 3. Comparison of cytokines, ghrelin and nesfatin-1 levels between controls with patients with brucellosis before and after treatment

	After 45 days of treatment with			
Variables	**Control**	**Patients**	**(DOX-STR)**	**(DOX-RIF)**
IFN-γ (pg/mL)	9.52 ± 8.5	16.71 ± 9.5*	11.53 ± 7.7*,**	12.97 ± 8.7*,**
IL-10 (pg/mL)	7.64 ± 5.4	11.36 ± 7.5*	8.47 ± 4.3*,**	9.96 ± 5.6*,**
Ghrelin acylated (pg/ml)	41.52 ± 19.41	82.45 ± 10.75*	58.57 ± 12.93*,**	67.23 ± 8.64*,**
Ghrelin deacylated (pg/ml)	474.53 ± 167.12	552.61 ± 132.51*	515.83 ± 110.32*,**	532.61 ± 158.81*,**
Total ghrelin	516.05 ± 124.58	653.06 ± 224.47*	574.50 ± 154.48*,**	599.84 ± 135.86*,**
Nesfatin-1 (ng/ml)	1.43 ± 0.36	0.89 ± 0.25*	1.27 ± 0.42*,**	1.13 ± 0.37*,**

Results are represented as mean ± SD, *P < 0.001, significant compared with controls; **P < 0.001, significant compared with patients

capacity may play a role in the pathogenesis of brucellosis [6]. Otherwise, IL-10 also inhibits the synthesis of pro-inflammatory cytokines such as TNF-α and IFN-γ [7].

In the present study, these cytokines (IFN-γ and IL-10) were analyzed and their levels significantly increased in patients with brucellosis compared with healthy subjects. Numerous studies have examined these biomarkers of inflammation in acute brucellosis. Many studies have shown high IFN-γ levels in patients with brucellosis compared with controls [3,8]. IL-10 levels also have been investigated and no difference was found in several studies, but Tang et al. [8] found that IL-10 levels were higher in acute brucellosis than in controls. The results confirm previous data showing that IFN-γ and IL-10 are involved in the immune response to brucella.

There are many various metabolic functions of ghrelin and nesfatin-1 to regulate appetite, food intake, energy expenditure, and body weight in the short and long term. Recent studies have revealed the functional roles of ghrelin and nesfatin-1 in the immune system, inflammatory stress and disease pathogenesis. In the immune system, ghrelin mRNA expression has been identified in lymphoid organs and B, T, natural killer (NK) cells, monocytes of human and rodent [9,10]. Dixit et al. [11] have reported the production and secretion of acylated/desacylated forms of ghrelin in T-cell activation. Immune system cells can sense changes in their environment and are affected by changes in circulating hormones and nutrients, and these cells can then influence immune responses and cytokine expression. Pro-inflammatory cytokines produced during inflammation also influence neuroendocrine function and metabolism. Experimental studies have shown that ghrelin levels are associated with the expression of pro-inflammatory cytokines, and exogenously administered ghrelin suppresses the expression/production of these cytokines in inflammation. Recent data have reported that ghrelin levels are increased in many inflammatory diseases such as ulcerative colitis, Crohn's disease (CD), rheumatoid arthritis, pancreatitis, and spondylitis [6].

Kilic et al. [12] observed that the serum ghrelin levels significantly decreased in brucella patients compared with controls. There were no other studies in the literature. In this study, serum ghrelin levels were significantly higher in brucella patients compared to healthy individuals. This finding was consistent with various studies that have shown an increase in serum ghrelin levels in inflammatory diseases.

Studies over the past decade have identified nesfatin as a potent antioxidant and anti-inflammatory mediator. Inflammatory diseases lead to the activation of neutrophils, increased production of pro-inflammatory cytokines and overgeneration of reactive oxygen species. The levels of nesfatin in these diseases have been examined, and one of which is diabetic neuropathy (DN). It has been shown that nesfatin-1 inhibits overproduction of ROS in PC12 cells following high-glucose exposure, in vitro model for DN [13]. In chronic gastric ulcers, it also promoted the healing by partially reversing the down-regulation of superoxide dismutase mRNA [14]. Myocardial infarction (MI) often causes oxidative stress and inflammation, and plasma nesfatin-1 levels were found significantly lower than in healthy controls [15]. Intraperitoneal administration of nesfatin-1 had a protective effect by lowering pro-inflammatory cytokines against isoproterenol induced MI in rats [16]. Also, nesfatin-1 ameliorated the experimental renal ischemia-reperfusion injury in rats by decreasing malondialdehyde level and increasing SOD and catalase activities [17]. Nesfatin-1 exerts its antioxidant and anti-inflammatory effects by inhibiting the overproduction of ROS and maintaining the balance of oxidant/antioxidant systems, regulating the inflammatory cells by inhibiting neutrophil infiltration and decreasing the release of inflammatory mediators. According to the literature, this study is the first to report serum nesfatin-1 levels in patients with brucellosis. The levels of nesfatin-1 were significantly lower in pre-treatment brucella patients, and treatment of patients with antibiotic combination, especially (DOX-STR), will ameliorate their nesfatin-1 levels.

Conclusion

It is thought that the increment of ROS and the decrement of antioxidant capacity may play a role in the pathogenesis of brucellosis. In this study, the levels of the inflammatory biomarkers and serum ghrelin and nesfatin-1 were determined in brucella patients, and the recent data suggest that ghrelin and nesfatin-1 as an anti-inflammatory agent and immune-regulating hormones/cytokines may be valuable in the treatment of inflammatory diseases.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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